Evaluation of Novaluron as a Feed-Through Insecticide for Control of Immature Sand Flies (Diptera: Psychodidae)

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ABSTRACT The development and survival of sand fly *Phlebotomus papatasi* Scopoli (Diptera: Psychodidae) larvae fed feces of Syrian hamsters, *Mesocricetus auratus*, that had been fed a diet containing novaluron were evaluated. In total, six larval diets were used in sand fly larval bioassays. Four groups of larvae were fed feces of hamsters that had been maintained on a diet containing either 0, 9.88, 98.8, or 988 ppm novaluron. Two additional groups were fed a larval diet composed of equal parts composted rabbit feces and rabbit chow containing either 0 or 988 ppm novaluron. No pupation, hence no adult emergence, occurred when larvae were fed feces of hamsters that were fed diets containing novaluron. The mortality of sand flies fed feces of treated hamsters occurred during larval molts. The results of this study suggest that a control strategy using rodent baits containing novaluron to control phlebotomine sand flies and zoonotic cutaneous leishmaniasis may be possible.

KEY WORDS *Phlebotomus papatasi*, novaluron, sand fly control

Phlebotomine sand flies (Diptera: Psychodidae) are the vectors of the protozoan parasites that cause leishmaniasis, and they are notorious pests of humans. Worldwide, there are an estimated 2 million new cases of leishmaniasis annually, and an estimated 12 million people are currently infected (WHO 2007). Throughout Asia and North Africa, the sand fly *Phlebotomus papatasi* Scopoli is the primary vector of *Leishmania major*, which is the causative agent of zoonotic cutaneous leishmaniasis (ZCL).

Semifossorial rodents serve as the primary reservoir hosts of ZCL in arid and semiarid Old World foci. In these ZCL foci, which have high diurnal temperatures and low relative humidity, populations of sand flies aggregate in the burrows of the rodent hosts of *L. major*. Sand fly larvae and adults thrive in the microclimate within the burrows where the abundant organic debris serves as the food source for sand fly larvae. In Old World ZCL foci, the larvae of *P. papatasi* frequently have been recovered from animal burrows (Artemiev et al. 1972, Morsy et al. 1993).

The only historical successes in suppressing the transmission of *L. major* have involved the destruction of large areas of natural habitat to eliminate reservoirs and vector breeding and resting places (Faizulin 1980). The use of insecticides to control sand flies in Old World ZCL foci has not been successful because insecticide applications introduced into rodent burrows do not reach the microhabitats of adult and immature sand flies due to the length and complexity of the tunnels that make up the burrows (Seyedi-Rashti and Nadim 1973, Karapet'ian et al. 1983). The development of new, efficacious methods for the control of the vectors of ZCL is needed.

Phlebotomine sand fly larvae have been observed feeding on the feces of rodents (WHO 1968). Feed-through rodent baits that contain insecticides have been suggested as a novel method for sand fly larval control, and the feasibility of this method has been established using diflubenzuron, a benzoylphenylurea chitin synthesis inhibitor, to control larvae of *P. papatasi* (Mascari et al. 2007). The objective of this study was to assess novaluron, which also is a benzoylphenylurea chitin synthesis inhibitor, as a rodent feed-through to control sand fly larvae. The development and survival of *P. papatasi* larvae fed feces of Syrian hamsters, *Mesocricetus auratus*, which had been fed a diet containing novaluron was evaluated.

Materials and Methods

Feeding Protocol. Twelve Syrian hamsters were housed individually in micro-isolator cages. The maintenance of the hamsters and the experimental procedures of this research followed Animal Care and Use Protocol 05-074, which was approved by the Institutional Animal Care and Use Committee at Louisiana State University, Baton Rouge, LA. Research was con-

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ducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council Publication, 1996 ed.).

Four hamster diets were prepared by adding novaluron (98.8% active ingredient [AI], Makhteshim Agan Industries Ltd., Tel Aviv, Israel) to a meal form laboratory rodent diet (5001 Rodent Diet, LabDiet, PMI Nutrition International, Brentwood, MO). Novaluron was added to the meal form hamster diet to achieve four concentrations in the diet (0, 9.88, 98.8, and 988 ppm) and was thoroughly mixed.

Three hamsters were randomly assigned to each of the four diet groups (0, 9.88, 98.8, or 988 ppm novaluron). The initial body weight of the hamsters was measured on the day before the experiment. The body weight of hamsters in different diet groups was compared using analysis of variance (ANOVA), performed with the general linear model (GLM) procedure of SAS (SAS Institute 2001).

At 1200 hours each day for 9 d, each hamster was provided 25 g of their respective diet. The uneaten portion of the food was collected the next day at 1200 hours, and the daily food intake for each hamster was calculated. The daily dosages of novaluron that were ingested by the hamsters were calculated by multiplying the daily food intake by the concentration of novaluron in the hamster's diet. Both the daily food intake and the daily dosages of novaluron for individual hamsters were compared within hamster diet groups; daily food intake and the daily dosage of novaluron also were compared between hamster diet groups. Each comparison was performed using a repeated measures ANOVA, performed with the GLM procedure of SAS (SAS Institute 2001). The Tukey multiple comparison procedure was used to separate significantly different means.

The feces produced by each hamster were collected daily for 9 d. The feces were placed in uncovered glass vials and dried at room temperature for 7 d. Once dry, the feces were stored at −70°C until used.

Bioassay. A laboratory colony of sand flies was established at Louisiana State University by using specimens obtained from a long-standing colony of a Turkish strain of *P. papatasi* maintained in the Department of Entomology at the Walter Reed Army Institute of Research (Silver Spring, MD). The sand flies in the colony were reared using a larval diet composed of a dried and decomposed mixture of rabbit feces and rabbit chow (1:1) (Young et al. 1981). The colony was maintained in environmental chambers at 28°C, 90% RH, and a photoperiod of 14:10 (L:D) h.

Six larval diets were used in sand fly larval bioassays. The feces collected from hamsters on day 9 were pooled by treatments and crushed using a glass mortar and pestle. Four groups of larvae were fed feces of hamsters in each hamster diet groups. Two additional groups of sand fly larvae were fed the rabbit feces–rabbit chow larval diet containing either 0 or 988 ppm novaluron. This approach allowed comparisons between the survival of sand fly larvae fed feces of hamsters that had been fed diets without novaluron and the untreated rabbit feces–rabbit chow larval diet as well as comparisons between the survival of sand fly larvae fed feces of hamsters that had been fed diets containing novaluron and a larval diet treated directly with novaluron.

The larval bioassays were conducted according to the methods described by Mascari et al. (2007). A 0.1-g portion of the larval diets was transferred to the plaster surface of each bioassay vial. Ten second instars (13 ± 1 d old) were transferred to each bioassay vial and held in an environmental chamber at 28°C, 90% RH, and a photoperiod of 14:10 (L:D) h. Six bioassay vials were used for each of the six larval diet groups.

The larvae were observed under magnification daily. Larval mortality, defined as the lack of response to prodding with a blunt probe after 15 s, was recorded, and the larvae were observed for abnormal behavioral and morphological characteristics. Evidence of feeding, the presence of frass in the vials, and dark material in the guts of larvae also was monitored.

The percentage of survival of sand flies and the age of the sand flies at death in each larval diet group were compared with a repeated measures ANOVA performed with the GLM procedure (SAS Institute 2001). The Tukey multiple comparison procedure was used to separate significantly different means. The mean number of days until adult emergence for larvae fed each larval diet was compared using Student's *t*-test (SAS Institute 2001). The percentage of survival of sand flies fed feces of untreated hamsters and the untreated rabbit feces–rabbit chow standard larval diet also was compared using Student's *t*-test (SAS Institute 2001).

Results

Feeding Protocol. The mean body weight of the 12 Syrian hamsters was 136.0 ± 20.1 g, and the mean body weights of hamsters in the different hamster diet groups were not significantly different ($F = 0.57$, df = 3, $P = 0.65$). The mean daily food intake of the hamsters was 7.6 ± 1.7, 8.2 ± 1.7, 7.7 ± 1.3, and 7.6 ± 0.8 g for hamsters receiving diets containing 0, 9.88, 98.8, and 988 ppm novaluron, respectively, and it was not significantly different ($F = 1.00$, df = 3, $P = 0.40$). The estimated mean daily dosages of novaluron for hamsters were 0.6 ± 0.1, 6.2 ± 0.9, and 56.6 ± 7.7 mg/kg body weight for hamsters receiving 9.88, 98.8, and 988 ppm novaluron, respectively.

Bioassay. Larvae in each of the larval diet groups were observed feeding, and frass was found in each bioassay vial. The mean percentage of survival from second instar to adult for the sand flies in the untreated hamster feces larval diet group was 100%, and it and was not significantly different from the 98.3 ± 4.2% survival for sand flies in the rabbit feces–rabbit chow larval diet group ($t = -1.00$, df = 10, $P = 0.34$; Table 1).

Sand fly larvae that were fed feces of hamsters that had consumed diets containing novaluron and larvae

Table 1. Percentage of mortality and longevity of second instars (13 ± 1 d old) of *P. papatasi* larvae fed feces of Syrian hamsters that had been fed a diet containing 0, 9.88, 98.8, and 988 ppm, or in an aged rabbit feces–rabbit chow (1:1) larval diet containing 0 and 988 ppm novaluron

Larval diet group	Mortality (%)	Longevity (d)
Hamster feces		
988 ppm	100.0a	4.7 ± 1.9a
98.8 ppm	100.0a	4.9 ± 2.0a
9.88 ppm	100.0a	4.8 ± 1.7a
0 ppm	0.0b	N.A.
Aged rabbit feces–rabbit chow		
988 ppm	100.0a	4.4 ± 1.6a
0 ppm	1.7 ± 4.18b	N.A.

Data are mean ± SE of six replicates with 10 larvae per replicate. Values within a column followed by the same letter are not significantly different from each other ($P > 0.05$). N.A., not applicable.

that had been fed the rabbit feces-rabbit chow larval diet containing 988 ppm novaluron were ataxic, ceased feeding, and died before pupation (Table 1). The mean longevity of sand fly larvae fed feces of hamsters that had been fed 9.88, 98.8, and 988 ppm novaluron, or the rabbit feces–rabbit chow larval diet containing 988 ppm novaluron was not significantly different (Table 1).

Discussion

The quantity of food eaten by the hamsters in this study was not affected by the incorporation of novaluron in a powdered diet. This suggests that novaluron-treated diets are palatable to hamsters and that novaluron could be incorporated into baits for other rodents. Some important rodent reservoirs of *L. major* in parts of the Middle East and Asia, including *Rhombomys opimus* and *Meriones libycus*, are readily attracted to grain-based baits (Yaghoobi-Ershadi et al. 2000, Yaghoobi-Ershadi et al. 2005). In Sub-Saharan Africa, rodent reservoirs of *L. major* such as *Arvicanthis* spp., *Mastomys* spp., and *Tatera* spp. are granivorous and also could be targeted with treated baits.

Sand fly larvae fed feces of hamsters that had been fed a diet containing novaluron began to die at a time when the control sand flies were molting from the second to third instar. This observation is consistent with second instar spined soldier bugs, *Podisus maculiventris* (Say), that had been exposed to a novaluron-treated substrate, and later exhibited ataxia and died as larvae (Cutler et al. 2006). The mortality of second instar *Culex quinquefasciatus* Say and *Aedes aegypti* (L.) principally occurred during the larval stage when they were exposed to 1 ppb novaluron in water (Mulla et al. 2003, Su et al. 2003).

Previously, diflubenzuron was evaluated as a rodent feed-through for sand fly larvae (Mascari et al. 2007). Unlike the present findings with novaluron, second instars of sand flies that were fed feces of hamsters that had been fed diets containing diflubenzuron died during the larval-to-pupal molt.

The pharmacokinetcs of novaluron in mammalian systems make it an appropriate choice for use in treated rodent baits. Novaluron is of very low toxicity to mammals by ingestion and other routes of exposure (FAO 2005). After ingestion the majority of novaluron is eliminated unchanged in the feces (FAO 2005). Novaluron is persistent in the environment. In a rotational crop study where 100 g (AI)/ha was applied to soil, between 32 and 49% of the original compound was still present after 127–195 d (FAO 2005). The results of this study suggest that a control strategy using rodent baits containing novaluron to control phlebotomine sand flies and zoonotic cutaneous leishmaniasis may be possible.

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